

IDENTIFICATION OF ALLANTOIN IN *PROTEA COMPACTA* SEED

S. E. DREWES AND J. VAN STADEN

(Departments of Chemistry and Botany, University of Natal, Pietermaritzburg)

ABSTRACT

By means of I.R., N.M.R. and mass spectrometry it was shown that on a dry mass basis approximately two per cent of *Protea compacta* seed consists of allantoin.

UITTREKSEL

IDENTIFIKASIE VAN ALLANTOIN IN *PROTEA COMPACTA* SAAD

Deur middel van I.R., K.M.R. en massaspektrometrie is vasgestel dat op 'n droë massa basis ongeveer twee persent van die saad van *Protea compacta* uit allantoin bestaan.

INTRODUCTION

While investigating the effect of endogenous hormones on the germination of *Protea compacta* seed (Brown and Van Staden, 1973) it was observed that concentrated ethanolic extracts of embryo material, when stored at 5 °C, yielded large quantities of needle-like crystals. On a dry weight basis the embryos contained approximately two per cent of this crystalline material. The crystals were insoluble in practically all organic solvents and only slightly so in water. They did, however, dissolve in hot water but crystallised rapidly upon cooling.

Due to the relative abundance of the crystalline substance and the fact that it may play an important role in the physiology of the seed, its identification was undertaken.

MATERIAL AND METHODS

Embryo material of *Protea compacta* R.Br. was homogenized with 80% ethanol, using approximately 25 ml of ethanol per gramme dry mass of material, and was then shaken for 24 hours at 18 °C. After filtering the extract in a Buchner funnel, the residue was thoroughly washed with 80% ethanol. The combined ethanolic filtrates were concentrated to a small volume under vacuum at 35 °C. The concentrated extract was kept at 5° C for 48 hours during which period fine needle-like crystals formed. These crystals were recovered by filtration, purified by recrystallization from water and subsequently used for chemical identification. The crystalline material was optically inactive.

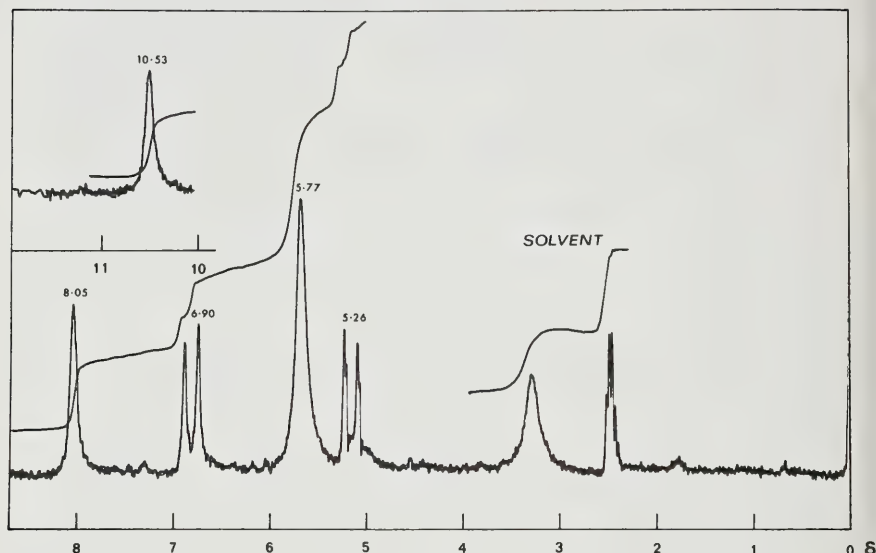


FIG. 1.
N.M.R. spectrum of crystalline compound from *Protea compacta* in $(\text{CD}_3)_2\text{SO}$.

Infra-red spectra were run as KBr discs, N.M.R. spectra were recorded on a Varian T60 instrument while a Varian CH7 instrument was used for mass spectrometry.

RESULTS

Using the above procedure every gramme of dry embryo material yielded 17 mg of a relatively pure crystalline compound which had a melting point of 235 °C. In water this substance showed no UV-absorption above 280 nm. Infra-red spectral analysis yielded the following significant data: ν_{nu} max 3438, 3340 (NH); 1780, 1705 (CO). The N.M.R. spectrum in $(\text{CD}_3)_2\text{SO}$ gives rise to:

- (i) a singlet at δ 10.53 (single proton -CO-NH-CO-),
- (ii) a singlet at δ 8.05 (single proton -HC-NH-CO-),
- (iii) a doublet at δ 6.90 (single proton -CH-NH-CONH₂),
- (iv) a singlet at δ 5.77 (two protons -CONH₂), and
- (v) a quartet at δ 5.26 (single proton -NH-CH-NH) (Fig. 1).

Low-resolution mass spectrometry of the unknown compound indicated a molecular ion of m/e 158. Major peaks were recorded at m/e 141 (M-17), 130 (M-CO), 115 (M-CONH), 87 (base peak), 60, 44 and 54 (Fig. 2).

Identification of Allantoin in *Protea compacta* Seed

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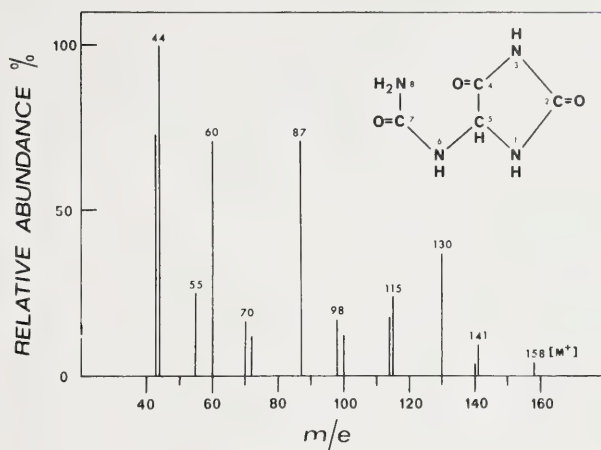


FIG. 2.

Mass spectrum of crystalline compound from *Protea compacta* seed identified as allantoin.

Elemental analysis gave the following results:

	Found	Calculated
C	30,48%	30,38%
H	3,87%	3,82%
N	35,63%	35,44%

The above analytical data yielded a molecular formula of $C_4H_6N_4O_3$. The physical data coupled with the fact that there was no depression of melting point when mixed with authentic material proves that the compound is racemic allantoin with the structure as indicated in Fig. 2.

DISCUSSION

The identification of allantoin in seed of *Protea compacta* adds to its already long history as a plant constituent. It was first isolated in 1881 by Schulze and Barbieri from sprouting shoots of *Platanus orientalis* and has subsequently been found in 22 families of flowering plants (Tracey, 1955). The fact that it is usually present in relatively high concentrations in plants suggests that it plays an important role in nitrogen metabolism. It would appear as if it is most abundant in xylem sap, ripening fruits, seeds and young developing leaves, flowers and seedlings (Tracey, 1955). All these are either organs in which large quantities of food reserves are stored or tissues which have a rapid rate of metabolism. Very

little definite information is, however, available regarding the physiological function of allantoin in plant material. Its frequent occurrence in seed led to the suggestion that it serves as a storage product for nitrogen (Mothes, 1958). It has also been postulated that it plays a role in the detoxification of ammonia, that it might be the form in which nitrogen is translocated in plants, or that it is a degradation product of purines (Mothes, 1958). The fact that it is present in *Protea compacta* seed where the major seed reserves are proteins ($\pm 65\%$), yet is absent in *Leucospermum reflexum*, a genus in the same family where the food reserve is lipid ($\pm 55\%$) (unpublished), suggests that it may act as a storage product in the former species. Since allantoin levels have been reported to increase during germination it may be that it is actively synthesized or alternatively it is formed as a result of purine degradation during this process (Barash, 1972).

The possibility of allantoin acting as an intermediate storage produce in the seed of *Protea compacta* is presently being investigated.

ACKNOWLEDGEMENTS

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